PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: YIMIN ZHAO, et al.

Serial No.: 10/673,030 Group No.:

Filed: September 26, 2003 Examiner.: --

For: QUERCETIN DERIVATIVES AND THEIR MEDICAL USAGES

Attorney Docket No.: U 014832-9

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sirs:

TRANSMITTAL OF ENGLISH TRANSLATION

OF PROVISIONAL APPLICATION

Attached is a Translator Signed English Translation of U.S. Provisional Patent Application No. 60/278,841.

Respectfully submitted,

JOHN RICHARDS
LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG. NO. 31053 (212) 708-1915

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450.

John Richards
(Type or print name of person mailing paper)

Date: January 26, 2004

(Signature of person mailing paper)

Certification

This is to certify that the attached document is a truly English translation of the US Provisional Patent Application No. 60/278,841.

Gesheng Huang,
Patent Attorney
Zhongzi Law Office

Signature: _____

Dated: Dec. 10, 2003

A Quercetin derivative and its medical usages

This invention relates to a quercetin derivative, the method of its preparation, and medicinal compositions containing this compound, as well as medical uses thereof for the prevention or treatment of diseases related to $5HT_{1A}$ receptor, especially for the prevention or treatment of depression and anxiety.

Glandless cottonseed, a variety of cottonseed, is the seed of glandless cotton, and normally used as feedstuff of livestock. Glandless cotton is a novel crop produced by selective breeding of cotton plant, a plant of the family Malvaceae. Up to now, the quercetin derivatives contained in cottonseeds (including glandless cottonseeds) and their biological activities have not been reported.

The purpose of this invention is to find out the biologically active chemical entities contained in cottonseed (including glandless cottonseeds), and to develop these chemical entities for medicinal uses.

Through extensive and intensive studies, the inventors have isolated the quercetin derivative shown in formula I. It was found that this compound could serve as a ligand of the $5HT_{1A}$ receptor. It exhibited favorable effects in treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, such as the effects of preventing gastric and duodenal ulcer, modulating heart function and blood pressure, as well as the effects on central neural disease such as depression and anxiety. This invention is achieved on the base of above discoveries.

the invention can be prepared according to known methods in the art, for example, by mixing the compound of formula I with pharmaceutical carriers.

According to the present invention, quercetin-3-O- α -D- apiosyl-(1→2)-[α -D-rhamnosyl-(1→6)]- β -D-glucoside of formula I is obtained, for example, from glandless cottonseeds. The organic solvents employed include alcohols, such as methanol, ethanol, propanol, butanol; halogenated hydrocarbons such as methylene dichloride, chloroform; esters such as methyl acetate, ethyl acetate, propyl acetate; and ethers such as petroleum ether, ethyl ether.

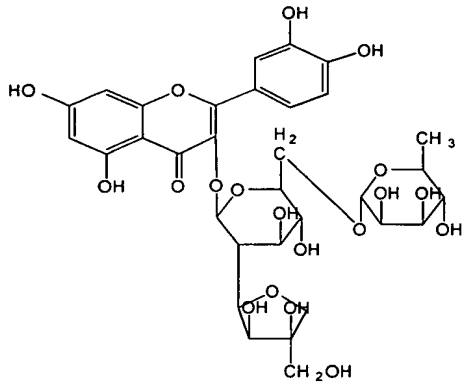
The following examples and bioactivity experiments further illustrate the present invention and are not intended to limit the invention in any way.

Example 1

Preparation of quercetin-3-O- α -D-apiosyl-(1→2)-[α -D-rhamnosyl-(1→6)]- β -D- glucoside

1kg of glandless cottonseeds are crushed and then passed through a 100 mesh, followed by 3 times of extraction with 5L petroleum ether for each time. The residues are then extracted 3 times with ethanol, 8L for each time. The extraction solution is then merged and evaporated under reduced pressure to constant weight. The 260 g of ethanol extract obtained is then dissolved in water, and distributed in n-butanol/H_2O solution, to give 10g of n-butanol extract and 200g of water extract. The n-butanol extract is separated through a silica gel column to give the compound of formula I, the developing agent is n-butanol:acetic acid:H_2O=7:1:2.

The compound of formula I thus obtained is yellow powder and turns into dark-red when heated in 10% EtOH-H_2SO_4, which indicates the existence of saccharide. Bright white fluorescence observed at 254nm, indicates itself being a flavonoid. Absorption peaks in IR spectrum (KBr) of said compound, of 3412cm^{-1}(ν_{-OH}), 2925 cm^{-1}, 1654 cm^{-1}($\nu_{-C=O}$), 1608 cm^{-1}, 1361 cm^{-1}, 1202 cm^{-1}, indicate existence of carbonyl and hydroxyl; the UV spectrum of said



Formula I

This invention relates to quercetin-3-O- α -D- apiosyl-(1 → 2)-[α -D-rhamnosyl-(1→6)]- β -D- glucoside, the compound shown in formula I. It can serve as a ligand of the $5HT_{1A}$ receptor, and has favorable effects in treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, especially in treating and preventing depression and anxiety.

The present invention further relates to pharmaceutical compositions comprising compound of formula I and pharmaceutical carriers.

The present invention further relates to the compound of formula I, for treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, especially for treating and preventing depression and anxiety.

The present invention further relates to pharmaceutical compositions containing the compound of formula I, for treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, especially for treating and preventing depression and anxiety.

According to the present invention, compound of formula I and pharmaceutical compositions thereof, of the present invention, can be administered orally, parenterally or tropically. The dosage form may be, for example, tablets, capsules, solutions, suspensions, injections and intravenous dripping solutions, etc.

According to the present invention, the pharmaceutical compositions of

compound: 256.2nm (log ε 3.95), 354.6nm (log ε 2.83), shows typical spectrum of a flavonol. The molecule weight of compound of formula I is determined to be 742 by FAB-MS. NMR data of compound of formula I is shown in table 1.

Table 1: ^{1}H-NMR and ^{13}C-NMR data of compound of formula I (400MHz)

Position	δ_H (J Hz)	δ_C
2		156.52
3		132.81
4		177.04
5		161.23
6	6.12 s	98.81
7		166.30
8	6.30 s	93.86
9		156.10
10		103.28
1'		121.87
2'	7.57 dd(2.0, 1.6)	115.24
3'		145.01
4'		147.00
5'	6.82 dd (8.48, 1.6)	115.80
6'	7.72 dd(8.48, 2.0)	120.80
1g	5.53 d(7.6)	100.70
2g	3.52 d(7.6)	76.99
3g		76.88
4g		70.36
5g		75.69
6g		66.88
1r	4.39 s	99.16
2r	3.11 s	70.30
3r		70.55
4r	3.09 s	71.84
5r		68.30
6r		17.78
1a	5.39 s	108.64
2a	3.84 br s	76.17
3a	---	79.33
4a	3.86 s	74.01
5a		64.34

Example 2

Experiments on the anti-depression activity of compound of formula I: quercetin-3-O- α -D-apiosyl-(1 → 2)-[α -D-rhamnosyl-(1 → 6)]- β -D-glucoside

1 Effect on the activities of adenylyl cyclase (AC) in rat cerebral cortex:

(1) Methods

Male Wistar rats weighed 200±20g are sacrificed by decollation, and cerebral cortex is separated. Synaptolemma is extracted at 4℃ according to the method as described in literature (Rasenick MM et al, Pro. Natl. Acad. Sci. USA, 1980; 77:4628) and suspended in buffer solution, so that a protein concentration of 3～5mg/ml is reached. The synaptolemma has to be incubated in advance with the test medicine, as adenylyl cyclase (AC) is located on it. The experiment is carried out as follows: portions of 100μl reaction solution containing certain concentration of test medicine and 15mmol/L HEPES, pH=7.5, 5mmol/L $MgCl_2$, 1mmol/L EGTA, 1mol/L DTT, 60mmol/L NaCl, 1mmol/L aminophylline, 0.5mg/ml phosphocreatine and 0.14mg/ml phosphocreatine kinase are respectively dispensed into reaction tubes, followed by addition of 20μg synaptolemma to each tube. Then, the tubes are immediately put into a water-bath at 30℃ to react for 10 minutes, which reaction is linear during the first 20 minutes. After that, all the reaction tubes are immediately transferred into boiling water and deposited for 3 minutes to terminate the reaction. The amount of cAMP thus produced is measured in an ice-bath environment with cAMP kit, the total reaction volume is 130μL. The measure is carried out according to the instruction of the kit: various reagents are added and, after the reaction finishes, the tubes are centrifugated at 4000 rpm for 7 minutes. 120μl supernatant is then pipetted into the measure cup, added afterwards with 1.5ml anhydrous alcohol, after shaken up, 3.5ml of scintillation solution is added. Then, the cups are sealed and shaken up, and are left overnight. The values of CMP of all samples are determined then by Wallac 1409 liquid scintillation counter. The amount of

cAMP produced can be calculated according to the standard curve and cpm value. The results are statistically analyzed by ANOVA, and Dunnett's T test is made for inter-group comparision. The results are shown in table 2 and table 3.

Table 2 Activation effects of imipramine and buspirone on AC

Medicines	Amount of cAMP produced (pmol/mg protein/minute)			
	25 μ M	100 μ M	400 μ M	1mM
imipramine	15.07±4.91	18.53±3.2*	30.32±5.63***	79.79±21.38***
buspirone	19.52±5.46*	19.71±5.57*	24.63±3.49***	33.00±8.58***
physiological saline	13.47±1.92	---	---	---

Table 3 Activation effect of compound of formula I on AC

Medicines	Amount of cAMP produced (pmol/mg protein/minute)			
	13.5 μ M	40.5 μ M	135 μ M	405 μ M
Compound of formula I	23.27±4.95*	4.75±6.33***	43.42±4.78***	68.34±10.45***
physiological saline	13.47±1.92	---	---	---

X±SD vs control group, *P<0.05, **P<0.01, ***P<0.001

(2) Discussion

It is indicated that anti-depression agent has an acute activation effect on synaptolemma AC, which might be an important step of its mechanism. It can be drawn from table 2 that typical anti-depression agent imipramine and atypical anti-depression agent buspirone dose-dependently activate AC. The compound of formula I remarkably activates AC, under a concentration of only 13.5 μ M (0.01mg/ml), up to 23.27±4.95 pmol/mg protein/minute. This effect is stronger than those of 25 μ M imipramine and buspirone. The activation effect of said compound at 404 μ M (0.3mg/ml) amounts up to 68.34±10.45 pmol/mg protein/minute, 2~3 times higher than same doses of imipramine and buspirone. Therefore, it can be concluded that compound of formula I has

an anti-depression effect with relatively higher activity.

2. Protection effect of compound of formula I on PC-12 cells damaged by corticosterone.

(1) Methods

PC-12 cells are diluted into a suspension (2×10^5 cells/ml) with DMEM culture solution containing 5% calf serum and 5% horse serum, and then are transplanted into 96-well plates pretreated with polylysine, and cultivated under conditions of 37℃ and 5% CO_2 for 2～3 days. Cells are to grow all over the wells bottom before test. The culture solution is then pipetted away and serum-free DMEM is added containing certain concentration of test medicine and 10^{-4} mol/L corticosterone, 10 μl of 5mg/ml MTT is added 48 hours later to each well, shaking up slightly and, 4 hours later, 100 μl of 10% SDS is added to each well, again shaking up slightly. The plates are then left in the incubator overnight at 37 ℃(about 8～12 hours). After all the dark-blue crystals are dissolved, shaking up slightly and absorbance (A) of each sample at 570nm is read using microplate reader. The results are then statistically analyzed by ANOVA, and shown in table 4.

Table 4 Protection effect of compound of formula I on PC-12 cells damaged by corticosterone

Medicines (μ mol.L^{-1})	Absorbance (A)	Increase of A (%)
Normal control	0.77±0.12	
Damaged control	0.24±0.04	
Compound of formula I		
4.04	0.74±0.14**	208.3
14.38	0.84±0.08***	250.0
40.43	0.86±0.10***	258.3
134.77	0.77±0.11***	220.8
404.31	0.61±0.16**	154.2

**P<0.01, ***P<0.001

(2) Discussion

Data in table 4 shows that the increase of A (%) of compound of formula I reaches as high as 208.3%, at a concentration of 4.04 μ mol/L. The higher the increase of A, the stronger the protection effect of said compound to PC-12 cells damaged by corticosterone. Therefore, said compound has a strong protection effect on PC-12 cells (rat pheochromocytoma cell strain) damaged by corticosterone, which is identical with the effect thereof on primary cultured hippocampal cells.

3 Forced swimming test

(1) Method

The test is carried out according to literature (Arch Int. Pharmacodn. Ther, 1977, 229(2): 327). 30 minutes after abdominal injection, the mice are put into an open glass box (19 cm high and 12 cm of diameter). Water inside the glass box is 8cm in depth and 22～23℃ in temperature. The mice are put into the water for 6 minutes and observed by Vidio movement analyzer, the accumulated immobility time of the mice during the last 4 minutes and their motility are statistically analyzed identically as above. The results are shown in table 5.

Table 5 Effect of compound of formula I on forced swimming behavior of mice

Medicines	Duration of immobility (sec)
physiological saline	184.94±19.15
Compound of formula I (mg/kg)	
0.31	148.69±30.81*
1.25	149.94±34.87*
5.00	134.38±40.99**

(2) Discussion

The forced swimming behavior test on mouse is a classical anti-depression model test. When the animal have been put in the water, it struggles and tries to escape at first, then enters a state of despair and becomes still and immobile. Anti-depressant can shorten the immobility time of the tested animal. Table 5 shows that compound of formula I can shorten the immobility time of mice at a dosage of 0.31mg/kg, therefore, the said compound has relatively high anti-depression activity.

4 $5HT_{1A}$ receptor test

(1) Method

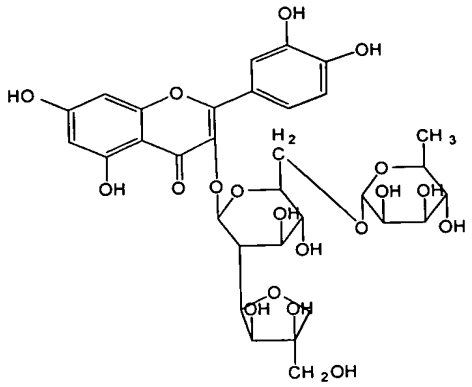
See table 6.

(2) Discussion

components of the assay solutions		total binding test (μL)	non-specific binding or competition test (μL)
labeled ligand	20nM ^{3}H-8-OH-DPAT	20	20
non-labeled ligand or tested samples	1mM 5-HT creatinine sulfate or the tested sample in different concentration	--	20
receptor membrane	1：5 suspension of rat hippocampal membrane	50	50
buffer	50mM Tris-HCl pH6.4	130	110
the above-mentioned assay solutions is mixed and then incubated in a water-bath at 25 ℃ for 30 minutes.			

Claims

1. The compound of formula I



2. Pharmaceutical compositions containing the compound of formula I and pharmaceutical carriers.

3. The compound of formula I or pharmaceutical compositions containing the compound of formula I which are used for prevention or treatment of diseases or symptoms related to $5HT_{1A}$ receptor.

4. Compound or pharmaceutical compositions according to claim 3, wherein the diseases or symptoms related to $5HT_{1A}$ receptor are depression or anxiety.

Abstract

This invention relates to a quercetin derivative, its preparation method, and pharmaceutical combinations containing this compound, as well as their medical uses for the prevention or treatment of diseases related to $5HT_{1A}$ receptor, especially for the prevention or treatment of depression and anxiety.